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RTI-31U-2796

DEVELOPMENT OF NEW PROPHYLACTIC RADIOPROTECTIVE AGENTS

Final Report

by

F. Ivy Carroll, Ph.D.

January, 1985
(For the Period 1 December 1983 to 31 December 1984)

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

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Research Triangle Institute
Research Triangle Park, NC 27709

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Radioprotection

S-2-(3-aminopropylamino)ethylphosphorothioic acid,

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The study presented in this report is concerned with the preparation of analogs of $H_2NCH_2CH_2NHCH_2CH_2SPO_3H_2$ (WR2721), with modifications in the terminal amine function as potentially new prophylactic radioprotective agents.

We have developed general methods for the synthesis of WR2721 analogs that possess amide [-C(=O)NHR] and thioamide [-C(=S)NHR] terminal residues. The

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method has been used to prepare the following six target compounds: $CH_3NHCO-CH_2CH_2NHCH_2CH_2SPO_3HLi$, $C_2H_5NHCOCH_2CH_2NHCH_2CH_2SPO_3HLi$, $CH_3NHCSCH_2CH_2NHCH_2CH_2-SPO_3HLi$, $C_2H_5NHCSCH_2CH_2NHCH_2CH_2SPO_3HLi$, $H_2NCOCH_2CH_2NHCH_2CH_2SPO_3HLi$, and $H_2NCS-CH_2CH_2NHCH_2CH_2SPO_3HLi$. Four of the above compounds were submitted to WRAlR for testing.

The intermediate, $BrCH_2CH_2NHCH_2CH_2C(=NH)NHCH_3 \cdot 2HBr$, required for the synthesis of the WR2721 analog, $CH_3NHC(=NH)CH_2CH_2NHCH_2CH_2SPO_3H_2$, was prepared and characterized.

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Foreword

The research project entitled "Development of New Prophylactic Radioprotective Agents" was the subject of an RTI proposal dated January 26, 1983 to the U. S. Army Medical Research and Development Command. The project was started December 1, 1983. H. A. Musallam of Walter Reed Army Institute of Research was the technical representative.

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1.0 Introduction

On January 26, 1983 we submitted a proposal entitled "Development of New Prophylactic Radioprotective Agents" to the U. S. Army Medical Research and Development Command. As a result of this submission, a contract (No. DAMD17-84-C-4002) for the period December 1, 1983 to December 31, 1984 was awarded to the Research Triangle Institute (RTI). The objective of the contract was to synthesize analogs of WR2721 with modifications in the terminal amine function.

This report presents the work carried out on this contract. Section 2.0 gives the background which formed the basis for the original proposal. Section 3.0 summarizes this synthesis work, and section 4.0 gives experimental details.

2.0 Background

Approximately 4400 compounds, mostly aminothiols and aminothiol precursors, were tested in mice in the 1959-1972 program of the U. S. Army.[1] Over 100 compounds were studied in larger animals, and a few were selected for IND applications.[2] The most effective radioprotective agent developed in the earlier U. S. Army program, S-2-[3-aminopropylamino]ethylphosphorothioic acid (WR2721), is the phosphorothioate of WR1065. The parent thiol, WR1065, has been shown to be active in radioprotection, and it is believed that WR2721 serves as a prodrug which releases WR1065 in tissue through the action of phosphatase enzymes.[3]

$H_2N(CH_2)_3NHCH_2CH_2SPO_3H_2$

WR2721

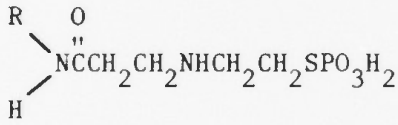
Comparison of WR1065 with its amide analog WR2529 (prepared at RTI) showed the two compounds to have essentially the same radioprotective activity.[1] However, whereas conversion of WR1065 to its phosphorothioate, WR2721, resulted in a less toxic and more active compound, derivatization of WR2529 gave a phosphorothioate, WR6458, which was more toxic and therefore could not be tested at dose levels comparable to WR2721. While these results are disappointing, it is surprising that no other amide derivatives were tested.[1]

$H_2NCH_2CH_2CH_2NHCH_2CH_2SH$ WR1065

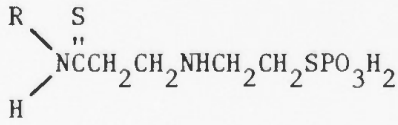
$H_2N\overset{O}{\overset{\|}{C}}CH_2CH_2NHCH_2CH_2SH$ WR2529

$H_2N\overset{O}{\overset{\|}{C}}CH_2CH_2NHCH_2CH_2SPO_3H_2$ WR6458

In 1972 the U. S. Army Research and Development Command decided to terminate efforts to develop radioprotective drugs. With the development of WR2721, many of the initial objectives had been met; however, all attempts to produce an orally active compound had failed. With the advent of increased drug development technology occurring between 1972 and 1978, the program was reestablished in 1978. One of the objectives of the new program was to initiate a limited drug synthesis effort to explore new and existing leads. In response to this objective, we are studying the synthesis of the amides T1, thioamides T2 and substituted amidines T3. These compounds can be viewed as analogs of WR2721 with modification in the terminal amine function.



T1



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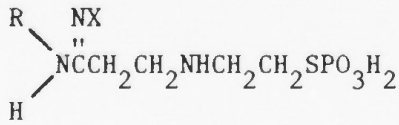


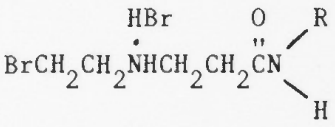
T3

3.0 Synthesis

3.1 S-2-[2'-N-Alkylcarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate and S-2-[2'-Carbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate

3.2 2'-[N-Alkylcarbamidoethylamino]ethyl Bromide Hydrobromide

2'-[N-Alkylcarbamidoethylamino]ethyl bromide hydrobromides (4) were key intermediates for the synthesis of the target amide phosphorothioates T1. In our proposal we indicated that the intermediates 4 could be prepared as shown in Chart 1. However, since this route involved the use of ethyleneimine and thus would have to be conducted in our toxic lab, we investigated alternate routes for the preparation of 4.



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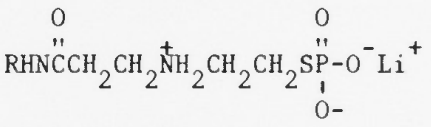


T1

Chart 2 outlines the first procedure we investigated. Treatment of acryloyl chloride (5) with two equivalents of methylamine or ethylamine gave the amide 2. The addition of excess ethanolamine to 2 gave mainly 6 contaminated with a small amount of $HOCH_2CH_2N(CH_2CH_2CONHR)_2$ which was easily separated by chromatography. When 6a was treated with thionyl bromide in 1,2-dimethoxyethane, the desired intermediate 4a was obtained. However, attempts to prepare large amounts of 4a led to an impure product. Fortunately, we were able to develop a modified procedure which can be used to prepare pure samples of 4a and 4b in large quantities. The new/modified route is shown in Chart 3. This route

Chart 1

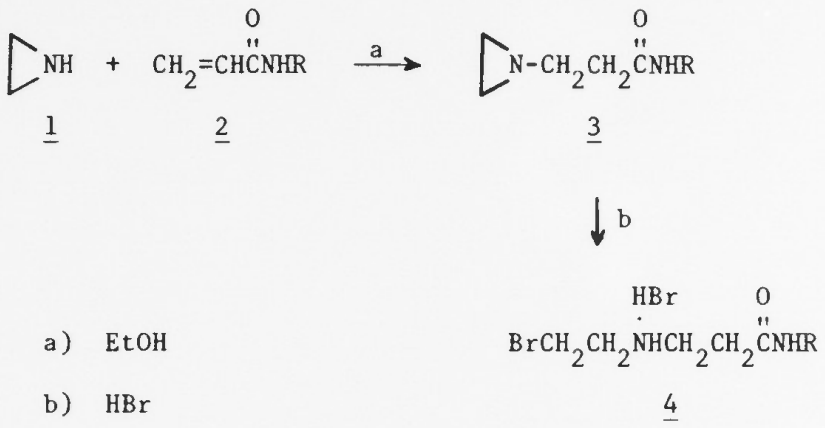


Chart 2

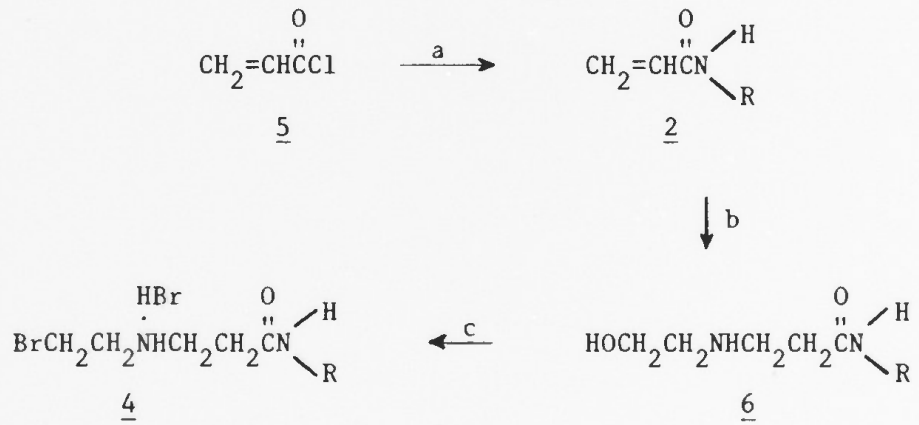
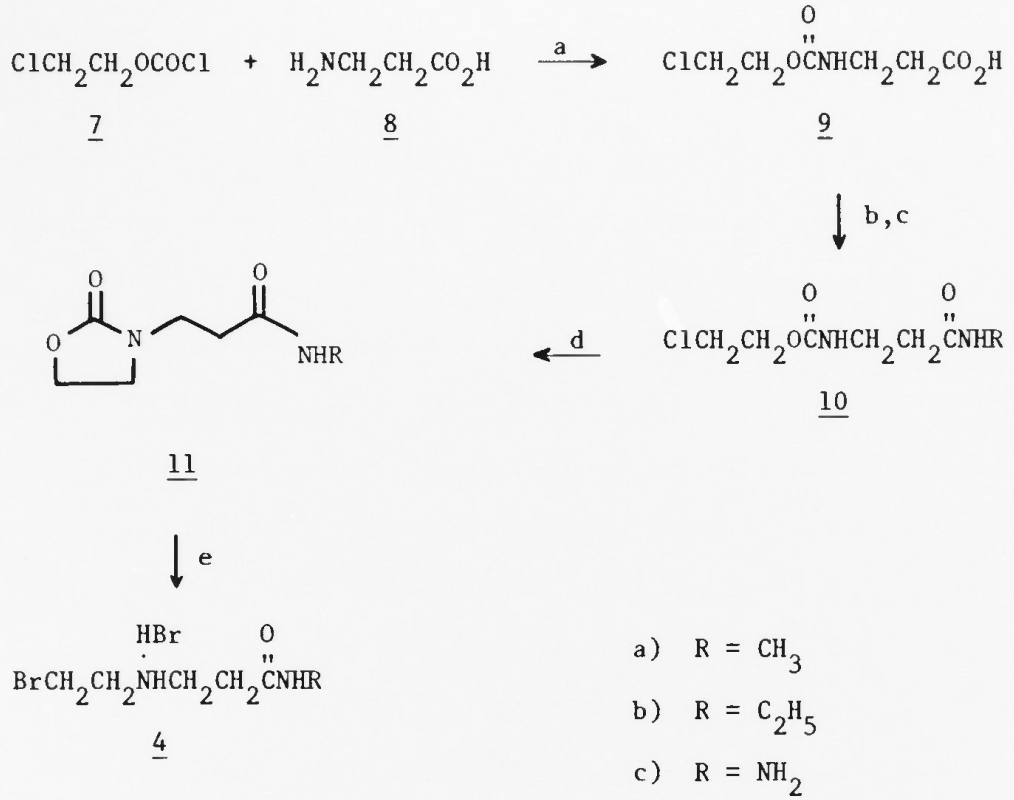


Chart 3



7 8 9 10 11 4

a) R = CH_3

b) R = C_2H_5

c) R = NH_2

a) NaOH

b) $SOCl_2$

c) RNH_2

d) NaH, DMF

e) HBr, HOAc

also has the advantage that most of the intermediates are crystalline solids that are easily purified by recrystallization. In the new method β-alanine (8) was condensed with chloroethyl chloroformate (7) to give the urethane 9. Reaction of 9 with thionyl chloride followed by treatment of the intermediate acid chloride with the appropriate amine gave the urethane amide 10. Base catalyzed cyclization of 10 using sodium hydride in DMF gave the oxazolone amide 11. Treatment of 11 with hydrogen bromide in acetic acid gave the desired bromo hydrobromide 4.

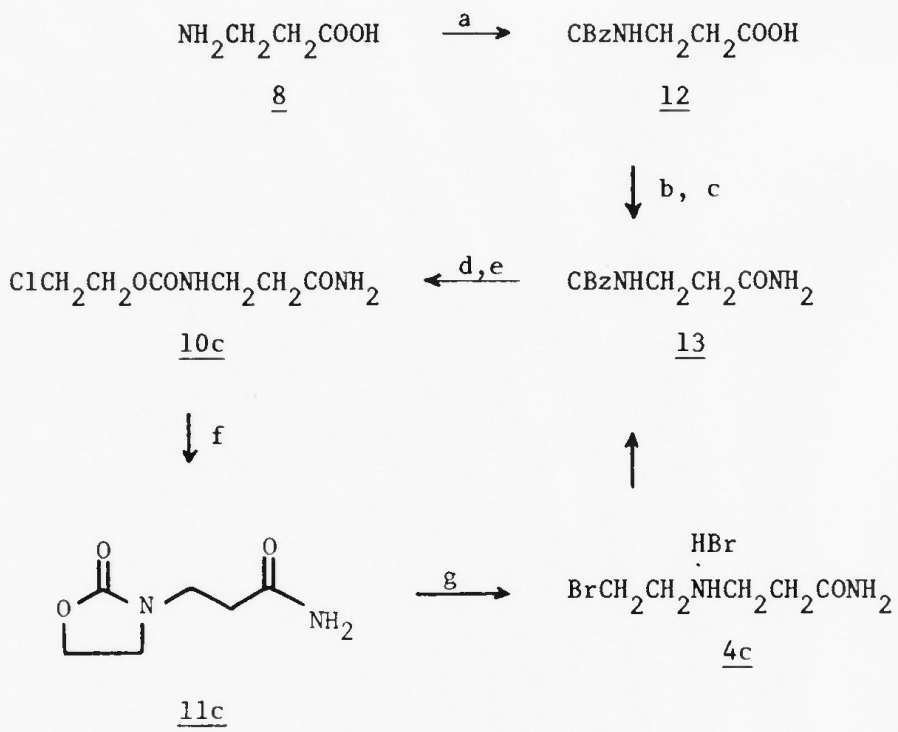
3.3 2'-(Carbamidoethylamino)ethyl Bromide Hydrobromide

Initially the unsubstituted bromide hydrobromide 4c was prepared as outlined in Chart 4. Thus, β-alanine was converted to the N-protected derivative 12 using carbobenzoxy chloride. Treatment of 12 with thionyl chloride followed by ammonia gives the amide 13. Subjection of 13 to catalytic hydrogenation followed by treatment of the liberated amine with chloroethyl chloroformate gave the intermediate 10c. Later we found that 10c could be prepared by a procedure exactly analogous to that used to prepare 10a and 10b (see Chart 3). Thus, treatment of 9 with thionyl chloride followed by the addition of two equivalents of ammonia gave 10c. Base catalyzed cyclization of 10c with sodium hydride in DMF gave the oxazolone 11c. Treatment of 11c with hydrogen bromide in acetic acid yielded the bromide hydrobromide 4c.

3.4 Phosphorothioate Reactions

The bromo hydrobromide 4a was first treated with trisodium thiophosphate to give the sodium hydrogen derivative 14 (Chart 5). The ^{31}P and ^{1}H NMR spectra of 14 were in complete accord with the proposed structure. However, the salt decomposed on drying under vacuum to give a product which showed several resonances in the ^{31}P NMR spectrum.

Chart 4



a) $CBzCl$, NaOH

b) $SOCl_2$

c) NH_3

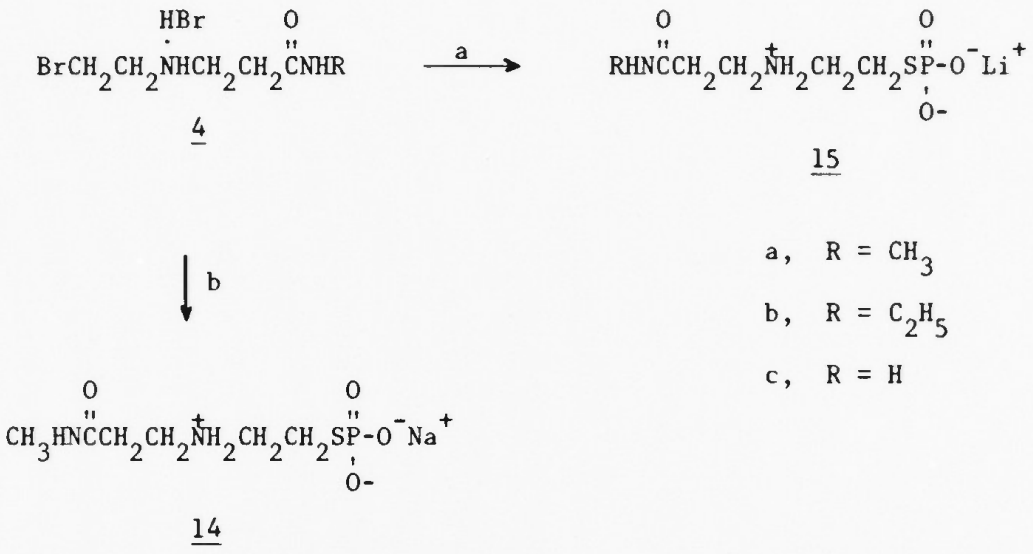
d) Pd/C, H_2

e) $ClCH_2CH_2OCOCl$, NaOH

f) NaH/DMF

g) HBr/AcOH

Chart 5



a) Li_3SPO_3

b) Na_3SPO_3

In contrast, treatment of an aqueous solution of 4a with trilithium thiophosphate followed by dilution with ethanol gave the lithium hydrogen salt 15a as a white stable solid. The product gave the expected ^{31}P and ^{1}H NMR spectra, but elemental analysis indicated the sample contained lithium bromide. In a separate experiment the product was isolated by dilution of the aqueous reaction mixture with dimethylformamide. Thorough washing with dimethylformamide and ether gave a solid which gave a good elemental analysis for 15a as well as the expected ^{31}P and ^{1}H NMR resonances. Similarly, treatment of 4c with trilithium phosphate followed by a dimethylformamide-ether work-up and washing gave a pure sample of 15c. Several attempts to prepare 15b by treating 4b with trilithium thiophosphate have yielded products which showed the expected ^{31}P and ^{1}H NMR spectra but gave incorrect elemental analyses.

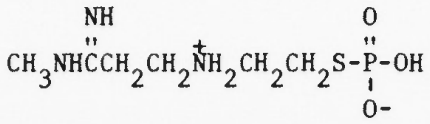
3.5 S-2-[2'-N-Alkylthiocarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate and S-2-[2'-Thiocarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate

Chart 6 outlines the procedure that was used to prepare the title compounds. Thiation of 11a-c with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-dithiophosphetane-2,4-disulfide (Lawesson reagent) in THF at 25°C gave the thioamides 16a-c. Treatment of 16a-c with hydrogen bromide in acetic acid yielded the bromo hydrobromide 17a-c. Phosphorothiation of 17a-c with trilithium thiophosphate using conditions similar to those used to prepare 15a-c gave the target compounds 18a-c.

3.6 S-2-[2'-N-Alkylamidinoethylamino]ethyl Dihydrogen Phosphorothioate

3.6.1 2'-[N-Methylamidinoethylamino]ethyl Bromide Dihydrobromide

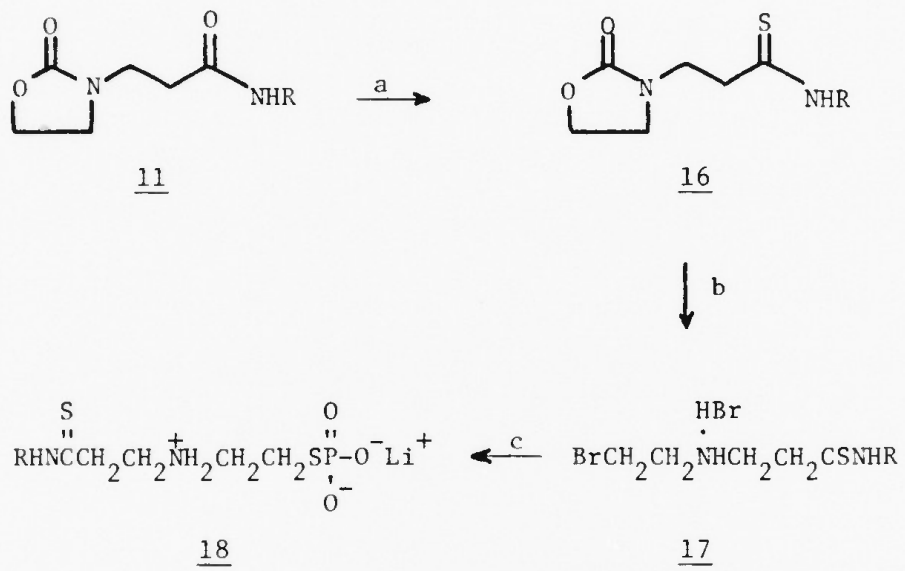
2-[N-Methylamidinoethylamino]ethyl bromide dihydrobromide (19) is an intermediate that is needed to prepare S-2-[2'-N-methylamidinoethylamino]-ethyl dihydrogen phosphorothioate (20). Chart 7 outlines the scheme



20

that was developed for the preparation of 19. Base catalyzed addition of oxazolidone (21) to acrylonitrile gave the adduct 22. Treatment of 22 with methanolic hydrogen chloride yielded the imino ester 23. The imino ester 23 was converted to the N-methylamidino derivative 24 by treating 23 with methylamine at -40°C. When 24 was dissolved in acetic acid saturated with hydrogen bromide, the desired bromo dihydrobromide 19 was obtained.

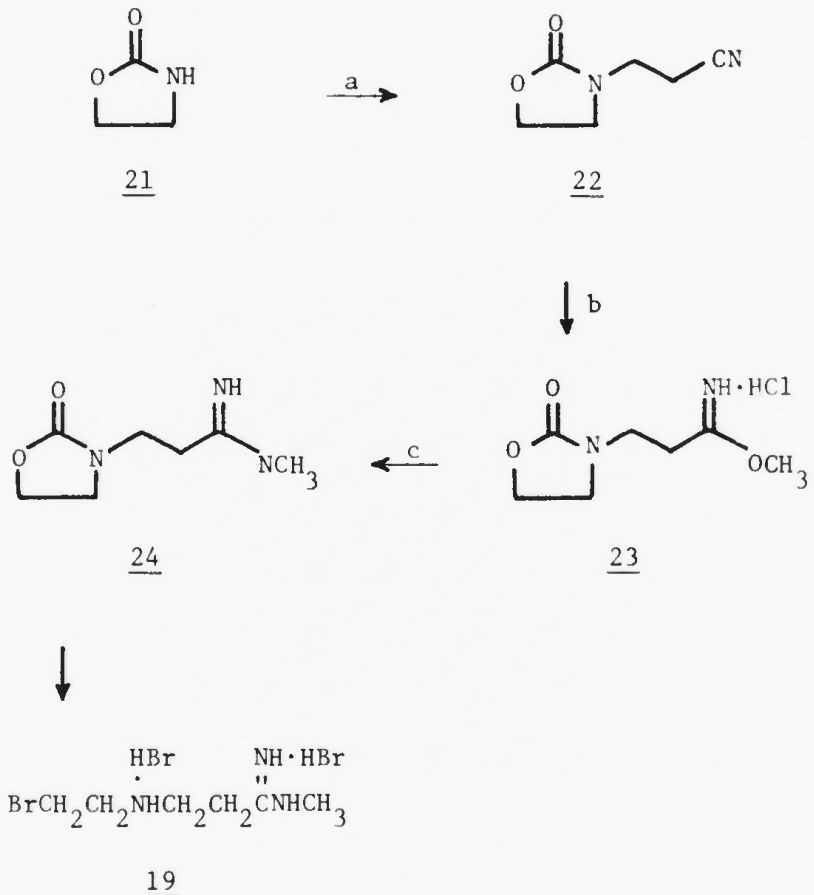
Chart 6



a, R = CH_3
b, R = C_2H_5
c, R = H

a) Lawesson Reagent, THF
b) HBr, HOAc
c) Li_3SPO_3

Chart 7



19

a) CH_2=CHCN, base

b) CH_3OH, HCl

c) CH_3NH_2

d) HBr, HOAc

4.0 Experimental Section

Melting points were determined on a Koffler hot stage. Infrared (IR) spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Ultraviolet spectra were run on a Varian model 2290 spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker 250 spectrometer. Chemical shifts were reported in δ values relative to tetramethylsilane (Me_4Si). Carbon and phosphorus magnetic resonance spectra were determined at 22.4 and 36.2 MHz, respectively, on a JEOL FX-90Q spectrometer.

N-Methylacrylamide (2a). Acryloyl chloride (45 g, 0.5 mol) was added dropwise to a stirred solution of 34.1 g (1.1 mol) of methylamine (generated from $CH_3NH_2 \cdot HCl$ with NaOH and distilled) in 100 mL of toluene at 0-5°C 2 h. After the toluene was removed under reduced pressure, the residue was distilled at 85-88°C (10 mm Hg) to give 19.9 g (48%) of 2a as a waxy solid; ^{1}H NMR ($CDCl_3$) δ 2.87 (2s, 3, NCH_3), 5.60 (m, 1, CH_2=C<u>H</u>-CO), and 6.28 (m, 2, <u>CH_2</u>=CH-).

2-(N-Methylcarbamidoethylamino)ethanol (6a). A solution of 2a (17.88 g, 0.21 mol) in 50 mL EtOH was added dropwise to a solution of ethanolamine (12.8 g, 0.21 mol) in 50 mL EtOH at 0-5°C over a period of 30 min. The reaction was allowed to rise to room temperature and then heated at 85-90° overnight. The solvent was evaporated under reduced pressure and the residue chromatographed on silica gel using $CHCl_3$:MeOH:NH_4OH (80:16:4) as eluant. The first fraction gave 2.09 g of bis-(2-N-methylcarbamidoethylamino)ethanol as a waxy solid. Further elution gave 27.9 g (90%) of 6a as a thick syrup; ^{1}H NMR (CD_3OD) δ 2.38 (t, 2, CH_2<u>CH_2</u>CO), 2.70 (s, 3, NCH_3), 2.71 (t, 2, $HOCH_2$<u>CH_2</u>N-), 2.85 (t, 2, $COCH_2$<u>CH_2</u>N), and 3.64 (t, 2, HO<u>CH_2</u>CH_2).

N-Ethylacrylamide (2b). Compound 2b was prepared in 20% yield as described for the preparation of 2a; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 3, $CH_2\underline{CH_3}$), 3.35 (q, 2, $\underline{CH_2}CH_3$), 5.56 (d, 1, CH_2=C$\underline{H}$-), and 6.31 (m, 2, $\underline{CH_2}$=CH-).

2-(N-Ethylcarbamidoethylamino)ethanol (6b). Compound 3b was prepared in 84% yield as described for the preparation of 6a; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3, $CH_2\underline{CH_3}$), 2.36 (t, 2, -$CH_2\underline{CH_2}$CO), 2.80 (t, 2, N$\underline{CH_2}CH_2$CO), 2.91 (t, 2, N$\underline{CH_2}CH_2$OH), 3.28 (q, 2, N$\underline{CH_2}CH_3$), 3.68 (t, 2, HO$\underline{CH_2}CH_2$-).

β-Chloroethoxycarbonyl-β-alanine (9). To a stirred solution of β-alanine (8) (44.5 g, 0.5 mol) and NaOH (20 g, 0.5 mol) in 500 mL of H_2O was added simultaneously chloroethyl chloroformate (71.5 g, 0.5 mol) and a solution of NaOH (20 g, 0.5 mol) in 100 mL of H_2O over a period of 1 h while the pH of the reaction mixture was maintained between 7-8. After stirring for 4 h at room temperature, the reaction mixture was extracted with ether. After acidification of the aqueous layer to pH 2-3 with 6N HCl, the mixture was extracted with ethyl acetate. The ethyl acetate solution was washed with NaCl solution and dried over Na_2SO_4. The residue after removal of the solvent was recrystallized from ethyl acetate/hexane to give 92.9 g (95%) of 9; mp 70-71°C; ^{1}H NMR ($CDCl_3$) δ 2.46 (t, 2, -CH_2CO_2), 3.47 (m, 2, NCH_2), 3.67 (t, 2, CH_2Cl), and 4.34 (t, 2, OCH_2).

Anal. Calcd for $C_6H_{10}N_2ClO_4$: C, 36.84; H, 5.15; N, 7.16; Cl, 18.12. Found: C, 36.94; H, 5.19; N, 7.16; Cl, 18.18.

N-Methyl-β-chloroethoxycarbamyl-β-alanine Amide (10a). A solution of 9 (50 g, 0.26 mol) in 350 mL of $CHCl_3$ was heated to reflux with $SOCl_2$ (61 mL) for 1 h. The solvents were removed under reduced pressure and dissolved in 50 mL of toluene and again evaporated to dryness under reduced pressure. A solution of methylamine (15.9 g, 0.52 mol) in

200 mL of $CHCl_3$ was added dropwise to a solution of the acid chloride in 350 mL of $CHCl_3$ at dry ice-acetone temperature over a perod of 1.5 h. When the addition was complete, the temperature was allowed to reach room temperature followed by stirring overnight. The precipitated methylamine hydrochloride was removed by filtration and washed with $CHCl_3$. The combined $CHCl_3$ solution was washed with saturated NaCl solution and evaporated to dryness. The resulting solid was recrystallized from $CHCl_3$/hexane to give 35.2 g (66%) of 10a; mp 125-126°C; ^1H NMR (CDCl3) δ 2.42 (t, 2, CH2CO), 2.81 (d, 3, NCH3), 3.46 (m, 2, NCH_2), 3.66 (t, 2, CH_2Cl) and 4.29 (t, 2, OCH_2).

Anal. Calcd for $C_7H_{13}N_2ClO_3$: C, 40.29; H, 6.28; N, 13.42, Cl, 16.99. Found: C, 40.23; H, 6.29; N, 13.41; Cl, 16.93.

N[-N-Methylcarboxamidoethyl]oxazolone (11a). To a suspension of hexane washed NaH [(50% suspension), 6.8 g, 0.16 mol] in 200 mL of dry DMF was added a solution of 10a (33.28 g, 0.16 mol) in 100 mL of DMF over a period of 1 h. The precipitated NaCl obtained after stirring overnight was separated by filtration, and the solution was evaporated to an oil under reduced pressure. The product was purified by silica gel column chromatography using 20% MeOH/CH_2Cl_2 as the eluant to give 16.5 g (94%) of 11a. ^1H NMR (CD_3OD) δ 2.44 (t, 2, CH_2CO), 2.71 (s, 3, NCH_3), 3.51 (t, 2, $NHCH_2$), 3.62 (t, 2, NCH_2 oxazolone) and 4.31 (t, 2, CH_2O).

Anal. Calcd for $C_7H_{12}N_2O_3 \cdot 1/4\ H_2O$: C, 47.58; H, 7.14; N, 15.85. Found: C, 47.23; H, 6.96; N, 15.59.

A 25.4 g (0.12 mol) run gave 16.2 (77%) of 11a.

2'-[N-Methylcarbamidoethylamino]ethyl Bromide Hydrobromide (4a). Method A (from 11a). A solution of 11a (13.5 g, 0.078 mol) in 20 mL of

acetic acid saturated with HBr was stirred at room temperature overnight. The excess HBr and some acetic acid was removed under reduced pressure. Dilution of the remaining solution with 10 mL of MeOH followed by careful addition of 100 mL of ether gave a slightly yellow precipitate. Filtration and recrystallization of the resulting solid from MeOH/ether gave 7.2 g (32%) of 4a; mp 124-126°C. ^{1}H NMR (CD_3OD): δ 2.67 (t, 2, CH_2CO), 2.75 (s, 3, NCH_3), 3.31 (t, 2, CH_2-N), 3.53 (t, 2, NCH_2) and 3.72 (t, 2, CH_2Br).

Anal. Calcd for $C_6H_{13}BrN_2O\cdot HBr$: C, 24.84; H, 4.86; N, 9.66; Br, 55.12. Found: C, 24.83; H, 4.89; N, 9.63; Br, 55.00.

A 0.06 mol run gave 68% of 4a.

Method B (from 6a). To a finely dispersed suspension of 6a (760 mg, 5 mmol)in 20 mL 1,2-dimethoxyethane at room temperature was added thionyl bromide (~2.1 g, 0.01 mol). The solution became dark red in color, and a vigorous reaction occurred. The reaction temperature was maintained between 45-60°C by external cooling and continued stirring for 30 min. After all the starting material had reacted (by TLC), the mixture was evaporated to a syrupy liquid and applied to a silica gel column (100 g) and eluted with 20% MeOH/CH_2Cl_2. The pure fractions were pooled together and evaporated to dryness to give 1.16 g of 4a as a glassy solid; NMR (CD_3OD) was identical to the sample prepared from 11a.

N-Ethyl-β-chloroethoxycarbonyl-β-alanine Amide (10b). Compound 10b was prepared by a procedure similar to that described for 10a. Thus, acid 9 (38.0 g, 0.2 mol) was converted to the acid chloride and treated with ethylamine (18.0 g, 0.4 mol). Work-up and recrystallization with $CHCl_3$/hexane gave 29.4 g (66%) of 10b; mp 129-131°C. ^{1}H NMR ($CDCl_3$): δ 1.14 (t, 3, CH_2CH_3), 2.40 (t, 2, CH_2CO), 3.29 (q, 2, CH_2CH_3), 3.47 (t, 2, NCH_2), 3.66 (t, 2, $ClCH_2$) and 4.29 (t, 2, OCH_2-).

Anal. Calcd for $C_8H_{15}ClN_2O_3$: C, 43.14; H, 6.79; N, 12.58; Cl, 15.92. Found: C, 43.24; H, 6.81; N, 12.56; Cl, 15.95.

N-[N'-Ethylcarboxamidoethyl]oxazolone (11b). Compound 11b was prepared by a procedure analogous to that described for 11b. Thus, 31.17 g (0.14 mol) of 10b was treated with 6.72 g (0.14 mol) of NaH (50% suspension) in DMF. After chromatography, 19.4 g (74%) of pure 11b was obtained as an oil. ^{1}H NMR (CD_3OD): δ 1.11 (t, 3, CH_2CH_3), 2.43 (t, 2, CH_2CO), 3.20 (q, 2, NCH_2CH_3), 3.51 (t, 2, $-CH_2N$), 3.63 (t, 2, NCH_2, oxazolone) and 4.31 (t, 2, $-CH_2O$); mass spectrum calcd for $C_8H_{14}N_2O_3$: 186.1004, found 186.1005.

Anal. Calcd for $C_8H_{14}N_2O_3$: C, 51.60; H, 7.58; N, 15.04. Found: C, 51.35; H, 7.65; N, 14.67.

This experiment was repeated on larger scales giving 74-84% yield.

2'-[N-Ethylcarboxamidoethylamino]ethyl Bromide Hydrobromide (4b). A solution of 11b (1.9 g) in 10 mL of acetic acid saturated with HBr was stirred overnight at room temperature. After removal of excess HBr and part of the acetic acid under reduced pressure, the mixture was diluted with 10 mL of MeOH and 100 mL of ether to give a white solid. The solid was collected by filtration, washed with ether and recrystallized from MeOH/ether to give 2.05 g (66%) of 4b; mp 127-128°C. ^{1}H NMR (CD_3OD): δ 1.13 (t, 3, CH_2CH_3), 2.67 (t, 2, $-CH_2CO$), 3.23 (q, 2, NCH_2CH_3), 3.34 (t, 2, $\overset{+}{N}CH_2-$), 3.52 ($BrCH_2CH_2\overset{+}{N}$) and 3.72 (t, 2, $BrCH_2CH_2\overset{+}{N}$).

Anal. Calcd for $C_7H_{14}Br_2N_2O$: C, 27.65; H, 5.30; N, 9.21; Br, 52.58. Found: C, 27.66; H, 5.32; N, 9.12; and Br, 52.49.

This experiment was repeated on a 0.03 mol scale to give 68% of 4b.

Carbobenzoxy-β-Alanine (12). To a stirred solution of β-alanine (22.5 g, 0.25 mol) and NaOH (10 g, 0.25 mol) in 100 mL of H_2O was added

carbobenzoxy chloride (42.5 g, 0.25 mol) and a solution of NaOH (10 g, 0.25 mol) in 100 mL H_2O simultaneously over a period of 1 h. The mixture was stirred for another 2 h. The unreacted carbobenzyloxy chloride was extracted with ethyl ether, and the aqueous layer was acidified with 6N HCl. The resulting white precipitate was separated by filtration, washed with H_2O and dried under vacuum. Recrystallization from EtOAc/hexane gave 3.5 g (80%) of 12; mp 96-97°C (Lit.:[4] mp 102-104°); ^{1}H NMR (CD_3OD) δ 2.49 (t, 2, CH_2CO) 3.36 (t, 2, NCH_2), 5.06 (s, 2, CH_2O) and 7.2-7.47 (m, 5, aromatics).

Carbobenzoxy-β-alanine Amide (13). A solution of (4.46 g, 0.02 mol) of 12 in 20 mL $CHCl_3$ was heated to reflux with 5 mL thionyl chloride for 1 h. The solvent and excess thionyl chloride was removed under reduced pressure. A solution of the residue in chloroform was added to a vigorously stirred solution of dry ammonia in THF (30 mL) at dry ice/acetone temperature. After 1 h, the temperature was allowed to rise to room temperature, and the solvent was removed under reduced pressure. The residue was washed thoroughly with water, dried under vacuum, and recrystallized from a $CHCl_3$/EtOH mixture to give 3.85 g (86%) of 13; mp 156-158°C (Lit.:[5] mp 164-165°C); ^{1}H NMR (CD_3OD) δ 2.44 (t, 2, CH_2CO), 3.41 (t, 2, NCH_2), 5.08 (s, 2, CH_2O), and 7.34 (s, 5, aromatic).

β-Chloroethyloxycarbonyl-β-alanine Amide (10c). Method A (from 10c). Compound 10c was prepared by a procedure similar to that described for 10a. Thus, acid 9 (65 g, 0.33 mol) was converted to the acid chloride and treated dropwise with a solution of NH_3 (11.3 g, 0.66 mol) in 30 mL of THF and stirred overnight. The mixture was diluted with water, and the organic layer was separated and washed with saturated sodium chloride solution and dried (Na_2SO_4). The residue obtained after removal

of the solvents was recrystallized from EtOAc to give 38.5 g (60 g) of 10c, mp 67-68°C. ^{1}H NMR (CD_3OD) δ 2.44 (t, 2, $CH_2\underline{CH_2}CO$), 3.41 (t, 2, $NC\underline{H_2}CH_2CO$), 3.68 (t, 2, $ClC\underline{H_2}CH_2O$) and 4.28 (t, 2, $ClCH_2\underline{CH_2}O$).

Anal. Calcd for $C_6H_{11}Cl_1N_2O_3$: C, 37.03; H, 5.70; Cl, 18.22; N, 14.39. Found: C, 37.12; H, 5.73; Cl, 18.12; N, 14.37.

Method B (from 13). A solution of 13 (33 g, 0.015 mol) in 50 mL MeOH and 50 mL THF was hydrogenated over 5% Pd/C for 1 h at which time the hydrogenolysis was complete. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The resulting residue was suspended and stirred in 50% MeOH/H_2O and treated with chloroethoxycarbonyl chloride (2.12 g) and a solution of NaOH (0.6 g in 4 mL H_2O) simultaneously over a period of 20 min. After 2 h, the mixture was extracted with EtOAc. The organic layer was washed with NaCl solution and dried (Na_2SO_4). The residue, after removal of the solvent, was recrystallized from EtOAc to give 2.2 g (75%) of 10c; mp 67-68°C. ^{1}H NMR (CD_3OD) was identical to a sample prepared by Method A.

N-Carboxamidoethyloxazolone (11c). Compound 11c was prepared by a procedure analogous to that described for 11a. Thus, 20.1 g (0.1 mol) of 10c was treated with 4.8 g (0.15 mol) of NaH suspension (50% oil suspension) in DMF. Work up and recrystallization from a $CHCl_3$/hexane mixture gave 12.6 g (77%) of 11c; mp 105-106°C. ^{1}H NMR ($CDCl_3$) δ 2.55 (t, 2, $CH_2\underline{CH_2}CO$), 3.57 (t, 2, $NC\underline{H_2}CH_2CO$), 3.70 (t, 2, $OCH_2\underline{CH_2}N$), and 4.33 (t, 2, $OC\underline{H_2}CH_2N$).

Anal. Calcd for $C_5H_{10}N_2O_3$: C, 45.56; H, 6.37; N, 17.71. Found: C, 45.62; H, 6.37; N, 17.70.

2-[Carbamidoethylamino]ethyl Bromide Hydrobromide (4c). Compound 4c was prepared by a procedure analogous to that described for 4a.

Thus, 11c (7.3 g, 0.046 mol) was stirred with hydrogen bromide in acetic acid (100 mL). Removal of the solvents under reduced pressure and recrystallization of the solid from MeOH/ether gave 8.23 g (60%) of 4c, mp 144°C. ^{1}H NMR (CD_3OD) δ 2.72 [t, 2, $CH_2\underline{CH_2}CO$-], 3.34 (t, 2, $NHCH_2$-CH_2CO), 3.52 (t, 2, $NHCH_2CH_2Br$) and 3.72 (t, 2, $NHCH_2\underline{CH_2}Br$).

Anal. Calcd for $C_5H_{12}Br_2N_2O$: C, 21.76; H, 4.38; N, 10.15; Br, 57.15. Found: C, 21.84; H, 4.38; N, 10.12; Br, 57.15.

S-2-[2'-N-Methylcarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate (15a). To a stirred solution of trilithium thiophosphate (0.696 g, 3 mmol) in 3 mL of H_2O was added solid bromo compound (4a). After all the solid dissolved, 1.5 mL DMF was added, and stirring was continued for 3.5 h. At this point ^{31}P NMR indicated that all the trilithium thiophosphate had reacted. A small amount of solid was removed by centrifugation. The clear supernatant was diluted with 30 mL DMF while maintained in a water bath at 20°C. The mixture was stirred for 30 min and again centrifuged. The supernatant was separated by decantation. The solid was stirred with DMF:ether (1:1) and centrifuged. The solid obtained was stirred with 200 mL ether and separated by filtration. This solid was dried under a stream of N_2 to give 0.47 g (63%) of 15a. ^{1}H NMR (D_2O) δ 2.67 (t, 2, $CH_2\underline{CH_2}CO$), 2.75 (s, 3, NCH_3), 2.96 (m, 2, $CH_2\underline{CH_2}S$), 3.24 and 3.27 (d t, 4, CH_2NCH_2); ^{31}P NMR (D_2O) 16.11 ppm (t, J = 13 Hz).

This experiment was repeated at 0.1 mol scale three times giving yields of 60, 80, and 88%.

Anal. Calcd for $C_6H_{14}H_2O_4PS \cdot 1/3\ H_2O$: C, 27.54; H, 5.97; N, 10.70; P, 11.84; S, 12.25. Found: C, 27.65; H, 5.80; N, 10.68; P, 11.95; S, 12.26.

S-2-[2'-N-Ethylcarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate (15b). The ethyl analog 15b was prepared by a procedure similar to that described for 15a. Thus, trilithium thiophosphate (0.89 g, 0.004 mol) and 4b (1.22 g, 0.004 mol) gave 0.90 g (88%) of 15b. ^{1}H NMR (D_2O) δ 1.12 (t, 3, CH_2CH_3), 2.74 (t, 2, CH_2CH_2CO), 3.05 (m, 2, SCH_2CH_2), 3.21 (q, 2, NCH_2CH_3), 3.33 and 3.38 (d t, 4, CH_2NCH_2); ^{31}P NMR (D_2O) δ 15.75 ppm (t, J = 13 Hz).

Anal. Calcd for $C_7H_{16}LiN_2O_4PS \cdot H_2O$, 0.7 LiBr: C, 24.69; H, 5.32; N, 8.21; S, 9.40; P, 9.07; Br, 16.40. Found: C, 24.43; H, 5.14; N, 7.92; S, 9.25; P, 9.06; Br, 16.10.

S-2-(2'-Carbamidoethylamino)ethyl Lithium Hydrogen Phosphorothioate (15c). To a stirred solution of trilithium thiophosphate (0.23 g, 0.001 mol) in 1 mL of water 0.31 g (0.0012 mol) 4c was added. After dissolution, 0.5 mL of DMF was added, and the mixture was stirred for 2 h. After successive addition of 5 mL of DMF and 4 mL of ether, the mixture was stirred for another 30 min. The solution was filtered, washed first with a mixture of DMF and ether (1:1, 50 mL) and finally with 150 mL of absolute ethanol. On drying in vacuo overnight 180 mg (76%) of 15c was obtained as a white solid. ^{1}H NMR (D_2O) δ 2.77 (t, 2, CH_2CH_2CO-), 3.5 (m, 2, -S-CH_2CH_2) and 3.4 (m, 4H, -CH_2-$\overset{+}{N}H_2CH_2$). ^{31}P NMR (H_2O) 15.63 ppm (t, J = 13 Hz).

Anal. Calcd for $C_5H_{12}LiN_2O_4PS$: C, 25.65; H, 5.17; N, 11.96; S, 13.69; P, 13.63. Found: C, 25.31; H, 5.25; N, 11.82; S, 13.57; P, 11.67*.

A 0.024 and run gave an essentially quantitative yield of 15c.

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* The phosphorus analysis was carried out 5 days after the C, H, N, S analyses were obtained. It appears that the sample was hydrated with 1.5 mol of water at this point.

N-[N'-Methylthiocarbamidoethyl]oxazolone (16a). A solution of 11a (13.1 g, 0.08 mol) in freshly distilled THF (300 mL) under N_2 was stirred for 2 h with Lawesson reagent (16.8 g, 0.04 mol). The mixture became a clear yellow solution, and a white precipitate began to appear. Part of the THF was removed under reduced pressure. The precipitate obtained from the cooled solution was separated by filtration to give 9.85 g of 16a. The product was recrystallized from MeOH/ether to give 9.0 g (62%) of 16a; mp 138-139°C. ^{1}H NMR ($CDCl_3$): δ 2.99 (t, 2, CH_2-CH_2CS), 3.16 (s, 3, NCH_3), 3.61 (t, 2, NCH_2CH_2CS), 3.71 (t, 2, NCH_2CH_2O) and 4.32 (t, 2, NCH_2CH_2O); mass spectrum calcd for $C_7H_{12}N_2O_2S$ m/e 188.0620, found 188.0622.

Anal. Calcd for $C_7H_{12}N_2O_2S$: C, 44.66; H, 6.42; N, 14.88; S, 17.03. Found: C, 44.06; H, 6.42; N, 14.64; S, 16.72.

2'-[N-Methylthiocarbamidoethylamino]ethyl Bromide Hydrobromide (17a). A solution of 16a (6 g, 0.032 mol) in 200 mL acetic acid saturated with hydrogen bromide was stirred overnight. The solution was concentrated under reduced pressure at 20-23°C. The hydrobromide salt that precipitated was collected and washed with ether and dried. Recrystallization from MeOH/ether gave 5.32 g (65%) of 17a, mp 116-119°C. ^{1}H NMR (CD_3OD) δ 3.1 (s, 3, CH_3), 3.15 (t, 2, CH_2CH_2CS-), 3.54 (m, 4, $CH_2\overset{+}{N}H_2CH_2$) and 3.75 (t, 2, CH_2CH_2Br).

Anal. Calcd for $C_6H_{14}Br_2N_2S$: C, 23.54; H, 4.61; N, 9.15; S, 10.48; Br, 52.22. Found: C, 23.63; H, 4.63; N, 9.13; S, 10.40; Br, 52.12.

S-2-[2'-N-Methylthiocarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate (18a). To a stirred solution of 0.23 g (0.001 mol) of trilithium thiophosphate in 5 mL of water was added 0.340 g (0.0012 mol) of 17a at room temperature. When all the solid was dissolved, DMF

(2.5 mL) was added and stirring continued for 2 h. The mixture was diluted with DMF (5 mL) followed by ether (20 mL). The thick jelly-like precipitate was stirred for 1 h. The partially granulated precipitate was washed with a 1:1 mixture of DMF and ether (50 mL) followed by ether (250 mL). The white solid was dried for 40 min to give 0.280 g (96%) of 18a. ^{1}H NMR (D_2O) δ 2.86 (t, 2, $CH_2\underline{CH_2}CS$), 3.02 (t, 2, $CH_2\underline{CH_2}$-S), 3.39 (t, 2, $N\underline{CH_2}CH_2CS$), 3.49 (t, 2, $\underline{CH_2}CH_2$-S). ^{31}P NMR (D_2O) 15.57 ppm (t, J = 13 Hz). This experiment was repeated on a 0.24 molar scale to give 94% of 18a.

Anal. Calcd for $C_6H_{14}LiN_2O_3PS_2 \cdot 1.5\ H_2O$: C, 24.74; H, 5.88; N, 9.61; P, 10.64; S, 22.01. Found: C, 25.00; H, 6.01; N, 9.71; P, 10.09; S, 22.29.

N-[N'-Ethylthiocarbamidoethyl]oxazolone (16b). Compound 16b was prepared in 50% yield by a procedure similar to that described for 16a. Thus, 18.6 g (0.1 mol) of 11b and 20.2 g (0.05 mol) of Lawesson reagent in THF was stirred overnight to give 9.8 g of 16b; mp 127-128°C. ^{1}H NMR ($CDCl_3$): δ 1.27 (t, 3, $CH_2\underline{CH_3}$), 2.97 (t, 2, $CH_2\underline{CH_2}CS$), 3.68 (m, 6, $\underline{CH_2}CH_3$, NCH_2, $N\underline{CH_2}CH_2O$) and 4.2 (t, 2, $NHCH_2\underline{CH_2}O$).

Repeating the experiment on a 0.04 mol scale gave 70% af 16b.

Anal. Calcd for $C_8H_{14}N_2O_2S$: C, 47.50; H, 6.97; N, 13.85; S, 15.84. Found: C, 47.33; H, 6.98; N, 13.83; S, 15.74.

2'-[N-Ethylthiocarbamidoethylamino)ethyl Bromide Hydrobromide (17b). A solution of 16b (2 g, 0.01 mol) in 5 mL of acetic acid saturated with hydrogen bromide was stirred overnight at room temperature. Excess hydrogen bromide and acetic acid was removed under reduced pressure. The resulting residue was dissolved in a minimal amount of MeOH and diluted with ether. The precipitate was collected, washed with

ether and recrystallized from MeOH/ether to give 2.43 g (76%) of 17b; mp 132-133°C. ^{1}H NMR (CD_3OD): δ 1.26 (t, 3, CH_2CH_3), 2.98 (t, 2, CH_2CH_2CO), 3.51 (t, 2, NCH_2CH_2), 3.57 (t, 2, CH_2CH_2N), 3.64 (q, 2, CH_2CH_3) and 3.75 (t, 2, $BrCH_2CH_2$).

A 0.03 mol scale run gave 68% of bromo derivative 17b.

Anal. Calcd for $C_7H_{14}BrN_2S \cdot HBr$: C, 26.26; H, 5.04; N, 8.75; S, 10.01; Br, 49.96. Found: C, 26.24; H, 5.06; N, 8.75; S, 9.99; Br, 49.90.

S-2-[2'-N-Ethylthiocarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate (18b). This compound was prepared using a procedure similar to that described for 18a. Thus, trilithium thiophosphate (0.230 g, 0.001 mol) and 0.358 g (0.0012 mol) of 17b gave 0.230 g (78%) of 18b. ^{1}H NMR δ 1.25 (t, 3, CH_2CH_3), 2.96 [m, 4, CH_2CH_2CS and SCH_2CH_2], 3.21 and 3.40 (2 t, 4, CH_2-N-CH_2) and 3.60 (q, 2, NCH_2CH_3). ^{31}P NMR (D_2O) 15.63 (t, J = 13 Hz).

This experiment was repeated on a 0.025 molar scale to give 90% of 18b.

Anal. Calcd for $C_7H_{16}LiN_2O_3PS_2 \cdot H_2O$: C, 28.38; H, 6.11; N, 9.46; P, 10.46; S, 21.65. Found: C, 28.02; H, 5.87; N, 9.30; P, 9.87; S, 21.47.

N-Thiocarboxamidoethyloxazolone (16c). A mixture of 25 g (0.17 mol) of 11c and 36.8 g (3.4 mol) of Lawessons reagent was stirred with 790 mL of freshly distilled THF for 2 h at which time the mixture became clear. This mixture on partial concentration under reduced pressure followed by dilution with hexane and cooling gave a precipitate which was collected by filtration and washed with cold ether. Recrystallization

from MeOH/ether gave 14.5 g (54%) of 16c, mp 141-142°C. ^{1}H NMR (DMSO-d_6) δ 2.69 (t, 2, $CH_2\underline{CH_2}CS$), 3.52 (m, 4, $\underline{CH_2}NHCH_2$) and 4.2 (t, 2, $CH_2\underline{CH_2}O$-).

Anal. Calcd for $C_6H_{12}N_2O_2S$: C, 41.36; H, 5.79; N, 16.08; S, 18.40. Found: C, 41.33; H, 5.82; N, 16.03; S, 18.46.

2'-[N-Thiocarbamidoethylamino]ethyl Bromide Hydrogen Bromide (17c). A mixture of 9.6 g (0.06 mol) of 16c and 100 mL of acetic acid saturated with HBr was stirred overnight at room temperature. The solvent was removed under reduced pressure to give a solid. Recrystallization from MeOH/ether gave 10.21 g (58%) of 17c, mp 116-118°C. ^{1}H NMR (DMSO-d_6) δ 2.92 (t, 2, $CH_2\underline{CH_2}CS$), 3.3 (t, 2, $N\underline{CH_2}CH_2CS$-), 3.48 (t, 2, $CH_2\underline{CH_2}N$) and 3.71 (t, 2, $CH_2\underline{CH_2}Br$).

Anal. Calcd for $C_5H_{12}Br_2N_2S$: C, 20.56; H, 4.14; Br, 54.82; S, 10.98. Found: C, 20.48; H, 4.15; Br, 54.82; N, 9.56; S, 10.95.

S-2-[2-Thiocarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate (18c). The target compound 18c was prepared by a procedure similar to that described for 18a. Thus, 0.23 g (0.001 mol) of trilithium thiophosphate and 0.330 g (0.0012 mol) of 17c gave 0.21 g (84%) of 18c as a white solid. However, elemental analysis shows 0.25 mol of LiBr. ^{1}H NMR (D_2O) δ 3.02 [m, 4, $CH_2\underline{CH_2}CS$ and $CH_2\underline{CH_2}S$], 3.41 and 3.50 (2 t, 4, $\underline{CH_2}N\underline{CH_2}$); ^{31}P NMR ($D_2O$) 15.75 (t, J = 13 Hz).

Anal. Calcd for $C_5H_{12}LiN_2O_3PS_2 \cdot 1/4$ LiBr $\cdot$ 1/2 H_2O: C, 21.38; H, 4.66; N, 9.97; P, 11.08; S, 22.82. Found: C, 21.42; H, 4.66; N, 9.88; P, 10.84, S, 22.37.

N-[β-Cyanoethyl]oxazolone (22). To a stirred mixture of 1.74 g (0.02 mol) 2-oxazolidone (21) in benzene (5 mL) and sodium hydroxide (160 μL) of 50% solution at 50-60°C was added acrylonitrile (1.06 g, 0.02 mol). After stirring for 2 h the cooled reaction mixture was

adjusted to pH 2 with concentrated H_2SO_4, and diluted with CH_2Cl_2 (50 mL). The resulting solids were separated by filtration. The filtrate was dried (Na_2SO_4) and evaporated to give 2.40 g (83%) of 22 as an oil. ^{1}H NMR ($CDCl_3$) δ 2.68 (t, 2, $CH_2\underline{CH_2}CN$), 3.57 (t, 2, $NC\underline{H_2}CH_2$), 3.75 [t, 2, $NC\underline{H_2}CH_2O$] and 4.39 (t, 2, $CH_2\underline{CH_2}O$-).

The reaction was repeated several times up to 0.5 molar scale giving 68-84% yield. The products obtained were sufficiently pure and used in the next step without further purification.

2'-[N-Methylamidinoethylamino]ethyl Bromide Dihydrobromide (19). Dry hydrogen chloride gas was passed through a solution of 22 (1.0 g, 0.007 mol) in MeOH (3 mL) at 0°C until saturation. The mixture was kept at that temperature for ~1 h and then allowed to reach room temperature overnight. The precipitated NH_4Cl was separated by filtration and the filtrate evaporated to dryness to give the 1.45 g methyl amidate 23 which contained ~10% of N-[N-methylcarbamidoethyl]oxazolone. ^{1}H NMR (CD_3OD) δ 2.29 [t, 2, $\underline{CH_2}$-(NH)C(OCH_3], 3.65 (t, 2, $NC\underline{H_2}CH_2$), 3.73 (t, 2, $NCH_2\underline{CH_2}O$), 4.17 (s, 3, OCH_3) and 4.39 (t, 2, $OC\underline{H_2}CH_2$).

To the above solid dissolved in MeOH (5 mL) and cooled to -40° - -50° was added 3 mL of methylamine. After warming to room temperature, the mixture was evaporated to a waxy solid without further purification. The residue was dissolved in acetic acid saturated with hydrogen bromide. After 16 h the mixture was concentrated to a small volume under reduced pressure without applying external heating. The resulting residue was washed with ether, and dried. Recrystallization from MeOH/ether gave 1.04 g (40%) of 19, mp 155° (dec). ^{1}H NMR (CD_3OD) δ 2.96 (s, 3, NCH_3), 3.05 [t, 2, $CH_2\underline{CH_2}C(NH)$], 3.49-3.64 (m, 4, $C\underline{H_2}N$-$C\underline{H_2}$) and 3.8 (t, 2, $CH_2\underline{CH_2}Br$).

Anal. Calcd for $C_6H_{16}Br_3N_3$: C, 19.48; H, 4.36; Br, 64.80; N, 11.36. Found: C, 19.54; H, 4.39; Br, 64.69; N, 11.34.

5.0 References

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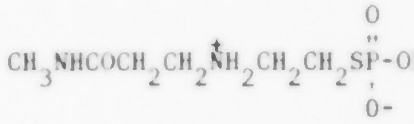
6.0 Appendix

Table of Compounds Submitted to WRAIR

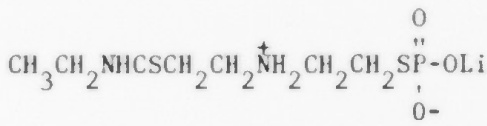
1. S-2-[2'-N-Methylcarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate



RTI-2796-1
15a from this report
target compound
7.1 g shipped

Synthesis is described on page 28.

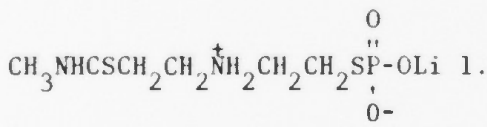
2. S-2-[2'-N-Ethylthiocarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate



RTI-2796-2
18b from this report
target compound
5.35 g shipped

Synthesis is described on page 32.

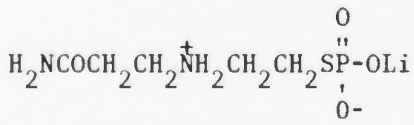
3. S-2-[2'-N-Methylthiocarbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate



RTI-2796-3
18a from this report
target compound
5.0 g shipped

Synthesis is described on page 30.

4. S-2-[2'-Carbamidoethylamino]ethyl Lithium Hydrogen Phosphorothioate



RTI-2796-4
18c from this report
target compound
5.0 g shipped

Synthesis is described on page 33.

7.0 Distribution

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